Appendix A

Replacement Specification Paragraphs and Amendments
to the Claims with Markings Showing Changes

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For: COMPOSITE DEVICES INCORPORATING BIOLOGICAL MATERIAL AND METHODS

All additions are indicated by underlining, all deletions are indicated by bracketing, and, additionally, all changes made in the specification paragraphs are highlighted.

IN THE SPECIFICATION

The two new paragraphs beginning at page 1, line 6

This application is a U.S. National Stage Application which claims priority to International Application No. PCT/US99/21581, filed September 17, 1999, which is incorporated herein by reference.

Statement of Government Rights

The present invention was made with support of the National Science Foundation under Grant No. 9424063, and the National Institute of General Medical Sciences under Grant No. T32-GMO-8347. The government may have certain rights in the invention.

The paragraph beginning at page 8, line 15

Other components (e.g., analytes in a sample of interest) that can be detected and/or quantitatively measured using the devices of the present invention include organic compounds that can be toxic to human, avian, plant, fish, insect, or other species. These include, for example, insecticides, herbicides, polycyclics, nerve gas agents, mutagens, carcinogens, antibiotics, products of combustion (e.g., tobacco smoke, coal combustion, liquid fuel combustion). Such compounds include**[,]** hydrocarbons (e.g., xylene, toluene, naphthalene), halogenated hydrocarbons (e.g., **[thrichloroethylene]** trichloroethylene, carbon tetrachloride, chloroform), formaldehyde, ketones, hydrazines, and the like.

The paragraph beginning at page 12, line 21

Preferably, the biological material includes one or more species of prokaryotic, eukaryotic, or archean organisms as homogeneous cell populations, mixtures of microorganisms, consortia, mixed-cultures, or unspeciated naturally occurring microbial populations with a defined characteristic. The biological material can include mammalian cells, blood cells, bacterial cells, avian cells, plant cells, insect cells, spores (e.g., *Bacillus subtilis*), phages (e.g., lambda bacteriophage), viruses (e.g., HIV, HTLV), etc. The biological material can be in the form of cell clumps or cell mats (i.e., a number of different cells living together **[is]** in some sort of structure), for example. Examples of suitable cells include bacterial cells such as *E. coli*, *Bacillus*, and *Streptomyces*, *Thermotoga*, archean cells such as *Pyrococcus*, eukaryotic cells such as yeast and *Penicillium*, as well as plant cells. For certain preferred embodiments, the biological material includes bacterial, yeast, or fungal cells, which may optionally be recombinant.

The paragraph beginning at page 25, line 16

Section B. Patches of immobilized *E.coli* HB101 harboring the pOS14 *mer-lux* constructs were analyzed individually for luciferase activity after exposure to $HgCl_2$ concentrations from 0.1 nM to 10,000 nM, **[At]** at 0.1 nM $HgCl_2$. Light induction was not significant compared to the control. Hg(II) concentrations at 1 nM and 10 nM induced luciferase activity after a 4-5 hour lag, and luciferase activity increased during the next 10 hours of the assay. At 100, 1,000 and 10,000 nM $HgCl_2$, luciferase activity was evident after 2 hours of incubation and reached the maximum detectable (6×10^6 count of single photons per minute) after 5-8 hours.

The paragraph beginning at page 27, line 6

Preparing Templates and Masks. A template is a pressure sensitive tape with sections cut out where the coating liquid is to contact the underlying coating or substrate. A template can be applied on top of a clean substrate or on **[a]** an already coated substrate. Masks are single pieces of pressure sensitive tape placed on top of the substrate or on coatings. After application of one or more layers on top of a template or mask each template or mask can be removed to expose the layer(s) beneath. Each template or mask was generated by manually punching with a ½ inch diameter punch (O'Brien Consolidated Industries, Lewiston, ME) or cutting with a razor blade. Templates **[was]** were generated by taping 5 pieces of pressure sensitive tape (clear vinyl) to the backside of a template figure and each section was cut through the 5 template pieces simultaneously on a poly-vinyl chloride board. The individual template sheets were separated and cleaned with KIMWIPEs to remove dust. Templates with nicks or tears were discarded since these would prevent them from separating from the substrate without tearing. Templates **[was]** were applied **[on to]** onto the substrate or coating by rolling **[it]** them onto it with a hard rubber roller (Orcon Corporation, Union City, CA). This method created uniform sections for patches or channels with a depth of 42.6 μm.

The paragraph beginning at page 28, line 7

Latex Biosensor Patch Shown in Figure 14. This device was created by coating a cell-latex mixture onto [a] an 8 hole ½ inch diameter template on a polyester substrate. The coating was then dried. A sealant coating was coated on top of the cell coating with the template still in place. Following drying of the sealant coating the template was removed leaving two layered patches of approximately 60 micron thickness on the substrate. A mask consisting of ½ inch circles were applied to each patch. A spacer was laid around the patches on each side to prevent contact between the [Mayor] Mayer rod and the masks during coating. A nonporous coating was subsequently coated on top of the masked patches and dried before removal of the masks. Induction at 100 nM Hg^{2+} resulted in a photon emission count of 500,000 counts per minute resulting from the mercury induced expression of luciferase. Induction at 0 nM Hg^{2+} resulted in less than 61 photon counts per minute.

The paragraph beginning at page 28, line 20

Latex Biosensor Patch Shown In Figure 15. This device was created by coating a cell-latex mixture onto [a] an 8 hole ½ inch diameter template on a polyester substrate. The coating was then dried. An absorbent coating was coated on top and dried. A sealant coating was coated on top of the absorbent coating with the template still in place. Following drying of the sealant coating the template was removed leaving three layered patches of approximately 90 micron thickness on the substrate. A mask consisting of ½ inch circles were applied to each patch. Surrounding the patches on each side a spacer was laid down to prevent contact between the [Mayor] Mayer rod and the masks during coating. A nonporous coating was subsequently coated on top of the masked patches and dried before removal of the masks. Induction at 100 nM Hg^{2+} resulted in a photon emission count of less than 1000 counts per minute resulting from the mercury induced expression of luciferase. The result demonstrated that the absorbent layer reduced the induced activity by 500 times. Induction at 0 nM Hg^{2+} resulted in less than 50 photon counts per minute.

The paragraph beginning at page 29, line 3

Latex Biosensor Patch Shown in Figure 16. This device was created by coating a cell-latex mixture onto **[a]** an 8 hole ½ inch diameter template on a polyester substrate. The coating was then dried. A sealant coating was coated on top of the cell coating with the template still in place. Following drying of the sealant coating the template was removed leaving two layered patches of approximately 60 micron thickness on the substrate. A second template consisting of ½ inch by 1 inch rectangular holes was applied on top of the **[of the]** patches so that 1/4 inch of **[the patch was covered]** the patches were covered and so that the open area connected to opposing patches. A porous channel layer was coated on top of the second template and dried. The second template was subsequently removed. A mask consisting of ½ inch circles **[were]** was applied to each patch. A spacer was laid around the patches on each side to prevent contact between the **[Mayor]** Mayer rod and the masks during coating. A nonporous coating was subsequently coated on top of the masked patches and the porous channel layer and dried before removal of the masks. Each patch with its channel was subsequently excised so that each patch had a ½ inch channel attached. 5 mm of the channel end was placed in induction buffer, leaving the circular part with cells out of direct contact with the induction buffer. Induction at 100 nM Hg^{2+} resulted in a photon emission count of than 50,000 counts per minute resulting from the mercury induced expression of luciferase. Induction at 0 nM Hg^{2+} resulted in less than 110 photon counts per minute.

IN THE CLAIMS

1. A composite biological device comprising a biostructure comprising at least one metabolically active biological material as an integral component thereof, wherein at least a portion of the biostructure comprises a nonporous latex-derived material.

2. The composite device of claim 1 wherein the biostructure comprises at least one layer comprising a porous latex-derived material and at least one layer comprising a nonporous latex-derived material.

3. The composite device of claim 1 wherein the nonporous material defines at least one channel or at least one well.

4. The composite device of claim 1 wherein the biostructure comprises no greater than about 75% by volume biological material.

5. The composite device of claim 4 wherein the biostructure comprises no greater than about 50% by volume biological material.

6. The composite device of claim 1 wherein the biological material comprises a procaryote, a eucaryote, an archean organism, or a combination thereof.

7. The composite device of claim 1 wherein the biological material comprises a mammalian cell, a blood cell, an avian cell, a plant cell, an insect cell, a bacteriophage, a spore, a virus, or a combination thereof.

8. The composite device of claim 1 wherein the biological material comprises a recombinant bacterial, yeast, or fungal cell.

9. The composite device of claim 8 wherein the recombinant cell is optimized for desiccation tolerance.

10. The composite device of claim 1 wherein the biostructure further comprises at least one additive selected from the group of a salt, a pigment, an adsorbent, a liquid crystal, a porosity modifier, a chelating agent, a nutrient, a surfactant, a dye, a photoreactive compound, an antibiotic, an antimicrobial, a bacteriostatic compound, an enzyme, an osmoprotectant, a biopolymer, a metal, a chemical catalyst, and a combination thereof.

11. The composite device of claim 1 wherein the biostructure further comprises a transmitter incorporated therein.

12. The composite device of claim 1 wherein the biostructure further comprises a detector incorporated therein.

13. The composite device of claim 12 wherein the detector senses a response emitted from the biological material when in contact with an analyte.

14. The composite device of claim 1 wherein the biostructure comprises a cross-linked latex-derived polymer.

15. The composite device of claim 1 wherein the biostructure is non-hydrated.

16. The composite device of claim 1 wherein the biostructure further comprises a porous latex-derived material.

17. The composite device of claim 16 wherein the porous latex-derived material comprises a mixture of latices.

18. The composite device of claim 1 further comprising a substrate on which the biostructure is disposed.

19. The composite device of claim 18 wherein the substrate comprises a membrane, a filament, or a wire.

20. The composite device of claim 18 wherein the substrate comprises a metal or a polymeric material.

21. The composite device of claim 18 wherein the substrate is an electronic device.

22. The composite device of claim 1 wherein the biostructure comprises wires or electrodes.

23. The composite device of claim 1 wherein the biostructure is no greater than about 500 microns thick.

24. The composite device of claim 1 wherein the entire device is no greater than about 500 microns thick.

25. A composite biological device comprising a 3-dimensional porous latex-derived biostructure comprising at least one metabolically active biological material incorporated therein; wherein the biostructure is disposed on a porous substrate.

26. A composite biological device comprising a 3-dimensional porous latex-derived biostructure comprising at least one metabolically active biological material incorporated therein; wherein the porous latex-derived biostructure comprises at least two portions of different pore size.

27. A method of making a composite biological device, the method comprising depositing at least one latex in a first layer; depositing at least one latex in a second layer on the first layer to form a multilayer microstructure; depositing at least one metabolically active biological material separately or in a combination with at least one latex such that the biological material is incorporated into the microstructure; wherein at least one of the latices forms a nonporous component of the microstructure.

28. The method of 27 wherein depositing comprises ink-jet printing with an ink-jet printer.

29. The method of 28 wherein the ink-jet printer includes piezo-electric or acoustic pumps.

30. The method of claim 27 wherein the second layer is deposited in a pattern.

31. A composite biological device for determining the presence of a metal in a sample, the device comprising a biostructure comprising at least one biological material, wherein, upon contact with the metal, the biological material produces a response and emits a signal.

32. The device of claim 31 wherein the biostructure comprises biological material immobilized in one or more layers of a polymeric material.

33. The device of claim 31 wherein the biological material comprises bacterial cells.

34. (AMENDED) The device of claim 33 wherein the bacterial cells comprise**[s]** *E. coli* cells.

35. The device of claim 31 wherein the biological material is genetically engineered to produce a response to the metal of interest.

36. The device of claim 35 wherein the biological material luminesces upon contact with the metal of interest.

37. The device of claim 36 wherein the biological material includes a metal resistant promoter and a reporter gene that encodes luciferase.

38. The device of claim 31 which is capable of detecting a metal in an inorganic or organic form.

39. The device of claim 31 which is capable of detecting mercury.

40. The device of claim 39 which is capable of detecting Hg^{2+} or monomethyl mercury.

41. The device of claim 31 further comprising a substrate on which the biostructure is disposed.

42. The device of claim 41 wherein the substrate is capable of detecting the signal.

43. The device of claim 42 wherein the substrate is a photosensitive film or a light-sensitive electronic chip.

44. The device of claim 41 wherein the substrate supports the biological material but does not detect the signal.

45. The device of claim 31 which is incorporated into a housing that is capable of penetrating a solid sample.

46. The device of claim 45 wherein the sample is mammalian, avian, or fish tissue.

47. The device of claim 31 which is capable of quantitatively measuring the amount of a metal in a sample.

48. A method of determining the presence of an analyte in a sample, the method comprises contacting the sample with the device of claim 1, wherein, upon contact with the analyte, the biological material produces a response and emits a signal; and detecting the signal.

49. A method of determining the presence of an analyte in a sample, the method comprises contacting the sample with the device of claim 31, wherein, upon contact with the analyte, the biological material produces a response and emits a signal; and detecting the signal.

50. (NEW) A microstructure device comprising:

an immobilized cell layer comprising cells capable of providing a detectable response to an analyte material present within a sample; and

a substrate for the immobilized cell layer.

51. (NEW) The microstructure device of claim 50, wherein the immobilized cell layer comprises a microporous polymeric matrix.

52. (NEW) The microstructure device of claim 51, wherein the cells are entrapped within the voids of the microporous polymeric matrix.

53. (NEW) The microstructure of claim 50, wherein the immobilized cell layer comprises cells that have been bio-engineered to produce the detectable response.

54. (NEW) The microstructure device of claim 50, wherein the detectable response comprises at least one selected from the group consisting of: fluorescence change, change in enzymatic activity, producing a metabolite, pH change, gas evolution, substrate consumption, color change, and mixtures thereof.

55. (NEW) The microstructure device of claim 50, wherein the substrate comprises means capable of detecting the detectable response.

56. (NEW) The microstructure device of claim 55, wherein the substrate further comprises an internal standard for the detectable response.

57. (NEW) The microstructure device of claim 55, wherein the substrate comprises a photosensitive film.

58. (NEW) The microstructure device of claim 55, wherein the substrate comprises a light sensitive electronic chip.

59. (NEW) The microstructure device of claim 50, wherein the substrate is either a substrate that transmits light or a substrate that blocks light.

60. (NEW) The microstructure device of claim 50, further comprising a protective film in contact with at least one of the immobilized cell layer and the substrate.

61. (NEW) The microstructure device of claim 50, further comprising a second polymeric layer.

62. (NEW) The microstructure device of claim 50, wherein the substrate comprises a monofilament that can be pulled through a sample.

63. (NEW) The microstructure device of claim 50, wherein the substrate comprises multiple filament threads that can be pulled through a sample.

64. (NEW) The microstructure device of claim 50, wherein the immobilized cell layer comprises cells selected from the group consisting of *Escherichia*, *Bacillus*, *Streptomyces*, and combinations thereof.

65. (NEW) The microstructure device of claim 50, wherein the immobilized cell layer comprises at least one polymer selected from the group consisting of an acrylate polymer, a vinyl acetate polymer, a styrene polymer, a butadiene polymer, a carboxylate polymer, and combinations thereof.

66. (NEW) The microstructure device of claim 50, wherein the immobilized cell layer comprises a polymer comprising a (acrylic-co-vinyl acetate) polymer.

67. (NEW) The microstructure device of claim 50, wherein the immobilized cell layer comprises glycerol.

68. (NEW) The microstructure device of claim 50, wherein the immobilized cell layer comprises at least one additive selected from the group consisting of an inorganic material, an amino acid, a pigment, an enzyme, a reactive dye, a photoreactive compound, a bacteriostatic compound, an antibiotic, an antimicrobial agent, an osmoprotectant, a biopolymer, a metal, a chemical catalyst, and mixtures thereof.

69. (NEW) The microstructure device of claim 50, wherein the analyte material comprises an environmental contaminant.

70. (NEW) The microstructure device of claim 50, wherein the sample comprises at least one of seafood tissue, sludge, and soil.

71. (NEW) A testing structure comprising:

an immobilized cell layer comprising cells engineered to provide a detectable response;

a substrate upon which the immobilized cell layer is provided; and

an interior volume, the microstructure device located within the interior volume, the interior volume configured to accept a sample to determine if the sample contains analyte material that will elicit a detectable response from the immobilized cell layer.

72. (NEW) The testing structure of claim 71, wherein the sample comprises a liquid contained within an absorbent material.

73. (NEW) The testing structure of claim 71, wherein the sample comprises a solid or semi-solid material.

74. (NEW) The testing structure of claim 71, wherein the sample comprises at least one of seafood tissue, sludge, and soil.

75. (NEW) The testing structure of claim 71, wherein the immobilized cell layer comprises a microporous polymeric matrix, wherein the cells are entrapped within voids present within said matrix.

76. (NEW) The testing structure of claim 71, wherein the detectable response comprises at least one selected from the group consisting of: fluorescence change, change in enzymatic activity, producing a metabolite, pH change, gas evolution, substrate consumption, color change, and mixtures thereof.

77. (NEW) The testing structure of claim 71, wherein the substrate comprises means capable of detecting the detectable response.

78. (NEW) The testing structure of claim 71, further comprising a protective film in contact with at least one of the immobilized cell layer and the substrate.

79. (NEW) The testing structure of claim 71, further comprising a second polymeric layer.

80. (NEW) The testing structure of claim 71, wherein the immobilized cell layer comprises cells selected from the group consisting of *Escherichia*, *Bacillus*, *Streptomyces*, and combinations thereof.

81. (NEW) The testing structure of claim 71, wherein the immobilized cell layer comprises at least one polymer selected from the group consisting of an acrylate polymer, a vinyl acetate polymer, a styrene polymer, a butadiene polymer, a carboxylate polymer, and combinations thereof.

82. (NEW) The testing structure of claim 71, wherein the immobilized cell layer comprises glycerol.

83. (NEW) A test device comprising:

an immobilized cell layer comprising cells bio-engineered to provide a detectable response;

a substrate upon which the immobilized cell layer is provided; and

a housing configured to sufficiently penetrate a solid object, the immobilized cell layer and substrate located within the housing.

84. (NEW) The test device of claim 83, wherein the housing is configured to penetrate a solid object comprising bodily tissue.

85. (NEW) The test device of claim 83, wherein the housing comprises a syringe, wherein an interior wall of the syringe bears the immobilized cell layer and substrate.

86. (NEW) The test device of claim 83, wherein the immobilized cell layer comprises a microporous polymeric matrix, wherein the cells are entrapped within voids present within said matrix.

87. (NEW) The test device of claim 83, wherein the detectable response comprises at least one selected from the group consisting of: fluorescence change, change in enzymatic activity, producing a metabolite, pH change, gas evolution, substrate consumption, color change, and mixtures thereof.

88. (NEW) The test device of claim 83, wherein the substrate comprises a material capable of detecting the detectable response.

89. (NEW) The test device of claim 83, further comprising a protective film in contact with at least one of the immobilized cell layer and the substrate.

90. (NEW) The test device of claim 83, further comprising a second polymeric layer.

91. (NEW) The test device of claim 83, wherein the immobilized cell layer comprises cells selected from the group consisting of *Escherichia*, *Bacillus*, *Streptomyces*, and combinations thereof.

92. (NEW) The test device of claim 83, wherein the immobilized cell layer comprises at least one polymer selected from the group consisting of an acrylate polymer, a vinyl acetate polymer, a styrene polymer, a butadiene polymer, a carboxylate polymer, and combinations thereof.

93. (NEW) The test device of claim 83, wherein the immobilized cell layer comprises glycerol.

94. (NEW) A test system comprising:

an immobilized cell layer comprising cells bio-engineered to provide a detectable response and a microporous polymeric matrix, the immobilized cells being entrapped within voids present within the matrix;

a substrate upon which the immobilized cell layer is provided;

a sampling device configured to penetrate a sample and obtain a testing sample; and

a testing volume containing nutrients and buffers suitable for the immobilized cell layer;

wherein a testing sample is obtained via the sampling device and is placed in the testing volume, the test device configured to determine if the sample contains an analyte material that will elicit a detectable response from the immobilized cell layer.

95. (NEW) The test system of claim 94, wherein the sampling device comprises a hollow coring device configured to remove a test sample, the immobilized cell layer and substrate being provided within the testing volume.

96. (NEW) The test system of claim 94, wherein the immobilized cell layer and substrate are located on the sampling device, the sampling device configured to place the immobilized cell layer in contact with the sample.

97. (NEW) The test system of claim 94, wherein the detectable response comprises at least one selected from the group consisting of: fluorescence change, change in enzymatic activity, producing a metabolite, pH change, gas evolution, substrate consumption, color change, and mixtures thereof.

98. (NEW) The test system of claim 94, wherein the immobilized cell layer comprises cells selected from the group consisting of *Escherichia*, *Bacillus*, *Streptomyces*, and combinations thereof.

99. (NEW) The test system of claim 94, wherein the immobilized cell layer comprises glycerol and a polymer comprising a (acrylic-co-vinyl acetate) polymer.